AN IN-SITU BIOASSAY STUDY TO INVESTIGATE THE ACUTE LETHALITY, HISTOLOGICAL DAMAGE AND THE BIOAVAILABILITY OF METALS TO FISH DOWNSTREAM OF TWO MINE-TAILINGS EFFLUENT DISCHARGES, MANITOUWADGE, ONTARIO

JULY 1996

Ontario

Ministry of
Environment
and Energy

ISBN 0-7778-5458-9

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PIBS 3448E

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ACKNOWLEDGEMENTS

The authors would like to acknowledge and thank the following contributors to this study:

i) Ian Smith - histological examination of the gills.

ii) Richard Chong-kit - gill tissue preservation and mounting in wax blocks and the photographing of the fish.

iii) Todd Kondrat - assisting in the field set-up.

iv) Jim Martherus + Gord Hitchin - fish checks + chemistry sampling when authors absent.

v) John Lee - for providing space for bioassays in Toronto lab.

vi) Al Smith (Norlab) - test fish holding & acclimation in Thunder Bay.

vii) MDS Medical labs - gill tissue sectioning, staining and mounting on slides.

viii) Ray Daust - for assisting in the electronic capture and presentation of the fish skin lesions in a pictoral format.

ix) Gary Westlake - manuscript review.

EXECUTIVE SUMMARY

This study was part of a project to describe the environmental effects of two mining operations on receiving waters in the Manitouwadge area. It documents the detrimental effects of acid mine wastes on fish as a result of: i) the primary seepages from both the Geco and Willroy mine properties into Fox Creek or directly into Manitouwadge Lake, and ii) the Noranda-Geco effluent discharge to Mose Lake.

The highly acidic leachate from the Willroy property contributed significant levels and loadings of metals to Harry's Creek, and produced a creek pH of 3.0 before entering Fox Creek. However, the main source of elevated metal levels in Fox Creek appears to be from the Geco mill complex and/or its' abandoned Glory Hole minesite. In turn, Fox Creek supplies the bulk of the metal loadings to Lake Manitouwadge due to its higher metal concentrations and flow rates relative to other potential sources. The other main source of metal loadings to the lake is the Camp Creek discharge from the Willroy tailings site, even though the Willroy mine has been inactive since 1977.

Field bioassays using rainbow trout were performed at various receiving water locations adjacent to the mine sites. Those tests measured acute fish lethality over an initial 96-h period, chronic toxicity involving the potential for histological damage to the surviving fish over a further 10-day exposure, with some of the fish analysed for the bioconcentration of heavy metals at the end of the study.

The runoff from the Willroy tailings area to both Camp Creek and Harry's Creek, was acutely lethal to rainbow trout. However, in both cases, the effect of those effluents on their receiving waters was localized. The excessively high pH levels (9.8-11.0) in the Camp Creek effluent was due to an ongoing effluent treatment process. However, the resultant effluent was adequately buffered by a downstream marsh, so that the final discharge to Lake Manitouwadge was non lethal. Also, the highly-acidic tailings leachate in Harry's Creek (3.0-3.5) was diluted at its' confluence with Fox Creek, thereby, increasing pH sufficiently to eliminate fish lethality beyond Harry's Creek.

All of the fish died in Noranda Creek, downstream of the effluent treatment plant of the Geco mine, within 96 h. The mortality was likely due to elevated un-ionized ammonia levels. It is possible that in the past, un-ionized ammonia could have approached lethal levels within the Mose Lake receiving water (ie. under the maximum possible ammonia, pH and water temperature conditions at that site). However, given the recently observed decreases in actual effluent ammonia concentrations, the elimination of ammonia use in the Geco mill in 1990, and the closure of the mine in 1995, ammonia

levels should continue their present decreasing trend to such a point that they will no longer remain an issue.

Chronic lethality was also observed during the study with 32% mortality observed in the sediment-exposed fish in Mose Lake at Noranda Creek, over a 15-day exposure. During the same period at that site, there was only 4% mortality in the fish caged at the water surface. The fish lethality that was observed in the sediment cage, may have been due to ammonia and/or the deposition of some other unidentified contaminant(s) from the Geco effluent. If the principal lethal component is heavy metals, the effects may not be as readily ameliorated as the ammonia problem in Noranda Creek.

There was no significant difference in the uptake of metals by fish exposed in Lake Manitouwadge at the Camp Creek discharge or by fish exposed in Mose Lake at the Noranda Creek discharge, relative to values measured in fish from the Fox Creek control. The bio-concentration of metals (copper, cadmium, zinc and manganese) was the highest for fish which were exposed to the sediments of Lake Manitouwadge at Fox Creek. The metal levels in those fish were significantly greater than the levels in fish exposed at the water surface of that site, as well as the levels in the control fish.

Skin lesions were observed on fish from a number of exposure sites during the study. No definitive cause could be determined but the possibility exists that the heavy metal exposures may have affected the immune system of the fish and made them more susceptible to a pathological or parasitic attack. A histological examination did not show any significant pathological changes to the gills of the fish sampled from any of the exposure sites over a 15-day exposure period during the study.

Laboratory bioassays were performed on grab samples of the Willroy tailings effluent discharged to Camp Creek at Dam H and the Geco Mine effluent at the weir. All samples (N=8) were acutely lethal in the rainbow trout and Daphnia magna tests and the fish bioassay results were consistent with the field results. Furthermore, these samples would not have met the fish lethality limit as set forth in the Metal Mining Regulation, which is scheduled to go into effect on August 25th, 1997. Un-ionized ammonia and elevated pH levels appeared to be the principal toxic components in those samples.

In conclusion, it appears that the Willroy and Geco effluents continue to produce a deleterious effect on their receiving waters. Therefore, the companies responsible for those mine sites should: 1) continue to treat their tailings until it can be demonstrated that their effluents are no longer acutely lethal to fish, nor would have a detrimental effect on the fishery, and 2) to provide a plan outlining the steps that they intend to take to address/ ameliorate the detrimental effects of the still untreated seepages originating from the Willroy property.

INTRODUCTION

1. Background

Base-metal mining and milling operations have been active in the Manitouwadge area since 1957. Those operations have resulted in a number of major environmental impacts due to metal contamination in the Manitouwadge chain of lakes. Contamination of the system is related to: i) past direct discharges and present seepage losses from the tailings site at the former Willroy mine, and ii) the wastewater treatment plant discharge, seepage losses and mine site runoff from current operations at the Noranda-Geco Mine (Figure 1).

Willroy Mines was a zinc-copper-lead mining and concentrator complex, which also processed ore from the Willecho and Nama Mines. The operation was active from 1957-1977 and reached a maximum production level of 2500 tonnes/ day. After it's closure in 1977, remedial measures were taken to stabilize the dam structures which impound the spent tailings. The tailings area was capped with clay and revegetated with grass. However, highly acidic seepages continue to flow from the site.

The main seepage flows south past dam A and into a collection pond. The pH of that pond is monitored and when values drop below 6.5, a 25,000 lb. slurry of calcium carbonate is added to the system. The overflow from the collection pond is then discharged over Dam H (Figure 1), and flows along Camp Creek, through the Camp Creek marsh and into Manitouwadge Lake.

There are also a number of secondary low-flow seepages along the eastern side of the tailings containment area (Dams D, G & F), which are uncollected and untreated. These seepages flow into Harry's Creek, which in turn, is assimilated into the much greater flow of Fox Creek, within 50 meters downstream of their confluence.

The Noranda-Geco complex is a copper-zinc-lead mining and milling operation which has a maximum production capacity of 4000 tonnes/ day and also began in 1957. There are a number of environmental concerns involving this operation and they are as follows:

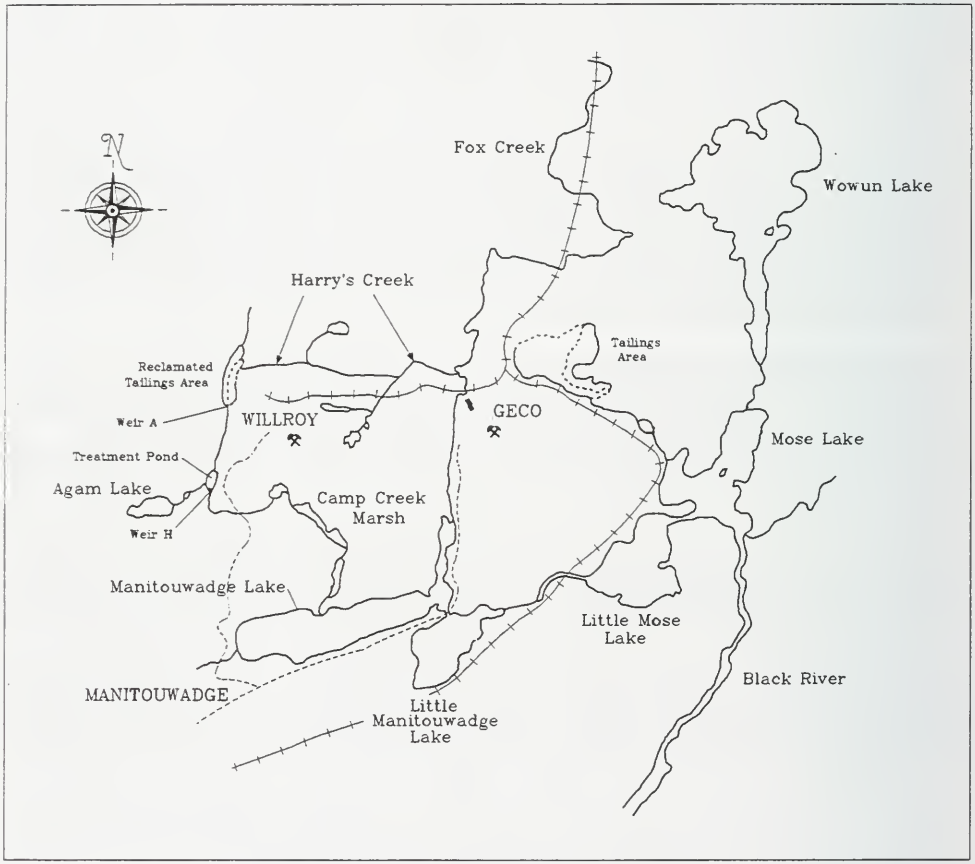
i) the presence of the highly acidic seepage from the tailings, which is typical of high sulphide ore bodies,

ii) historic seepage losses toward Mose Lake,

iii) present seepage losses to Fox Creek,

iv) contamination of drainage from the site, specifically, the former concentrate loading area (just downstream of the old railway bridge) and from the Glory Hole mineshaft, and

FIGURE 1: MANITOUWADGE STUDY AREA.



v) the previous use of ammonia for pH control during the milling process from 1957-1990, which has led to the saturation of tailings pore water with ammonia.

Although ammonia levels have decreased since the cessation of its' use, concentrations within the treatment plant continue to exceed 30 mg/L (Appendix B). Other process changes or treatment upgrades include the installation of a lime neutralization plant in 1976 to treat acid mine water and excess tailings effluent prior to discharge into Mose Lake. A major dam improvement and expansion program in 1992, to reduce or eliminate a number of seepages that were flowing directly into Fox Creek or Mose Lake. Also, subsequent to this study, the Geco wastewater treatment plant was upgraded to a 2-stage neutralization and high density sludge process in 1994.

There have been a number of environmental studies completed to date that have described the downstream impacts of mining on the water quality, benthos and sediment chemistry in the area (eg. German, 1972; Munkittrick and Dixon, 1989). However, given that Noranda-Geco has projected that they would likely undergo closure before 1996, and given the uncertainty that was associated with the actual level of acid-leaching impacts from the decommissioned Willroy property, it was determined that there was a need to better document the environmental effects of mining operations in the Manitouwadge area on the upper reaches of the Black River. In 1991, the Ontario Ministry of the Environment and Energy (OMEE) initiated a number of studies in an attempt to better quantify those impacts. The purpose of the present study was to determine whether there were any detrimental effects on fish that could be attributed to i) Noranda-Geco's final effluent discharge to Mose Lake, and/or ii) the primary seepages from the two mining properties to Fox Creek or directly to Manitouwadge Lake.

2. Study Area

The study area, which focused on Camp Creek, Fox Creek, Manitouwadge Lake and Mose Lake, is located at the upper reaches of the Black River system, which in turn, eventually discharges into Lake Superior, near Marathon, Ontario. Drainage from the Willroy property flows into Manitouwadge Lake from two locations. The main seepage flows south into a large dammed impoundment where calcium carbonate is added, and from there it flows through a flume into Camp Creek, through a marsh and into Manitouwadge Lake. A smaller continuous flow enters Harry's Creek on the eastern side of the tailings area, and flows eastward until it discharges into Fox Creek approximately 750m upstream of the Geco mill site. In turn, Fox Creek flows south to Manitouwadge Lake (Figure 1).

Drainage from the Geco property including the initial mine site, Glory Hole, flows into Fox Creek downstream of the mill site. The final mill effluent is treated with calcium carbonate and released

into Noranda Creek, which flows east from the mill complex, through marshlands, and over a number of dams before making its way to Mose Lake.

The continuous input of wastes from Noranda Creek, with its high dissolved solids content has produced a distinct chemical layer (chemocline) approximately 3-4 m below the lake surface which prevents Mose Lake from mixing adequately (German, 1972). It has resulted in major accumulations of aluminum, copper, zinc, lead and iron in the sediments, nearly anoxic conditions at the lake bottom and the total elimination of benthic invertebrates throughout the deeper sections of the lake.

METHODS

1. Study Outline

Field bioassays using hatchery-reared rainbow trout (Oncorhynchus mykiss) were performed at various receiving water locations adjacent to the Willroy and Geco Mine sites. Those fish exposures were undertaken to measure acute lethality over a 96-h period, beginning on September 16th, 1991 (study day 1).

Fish, which survived the 96-hour exposure, were left in the cages for an additional 10-day period. Upon completion of the field exposure, all surviving fish were sacrificed with a portion of them preserved in a 10% formalin solution in order to perform a histological examination of their gills. Any fish with external lesions were packaged individually, frozen and upon return to the Rexdale lab., they were photographed for future reference. The remaining fish were frozen and submitted for metals analyses.

During the initial 96-hour fish exposure, there were several rain events. Water samples were collected for metals analyses during each rain event in order to determine whether there were significant pulses of elevated metal concentrations during those periods.

Grab samples of the Willroy property runoff at Dam H and the Geco Mine effluent at the weir, were collected daily during the initial 96h of the field bioassay and shipped to the MOEE Rexdale lab for bioassay testing. The bioassays were undertaken in order to measure the acute lethality of those effluents to rainbow trout fingerlings and Daphnia neonates. Dechlorinated Toronto tap water was used to dilute the samples in order to determine an LC50 and 95% fiducial limits. Samples from some of the bioassay dilutions were analysed for ammonia and various heavy metals in an attempt to determine the component(s) which produced acute lethality in those samples.

2. In-situ Bioassay

a) Test Fish

Rainbow trout fingerlings were obtained from the Rainbow Springs hatchery in Erin, Ontario, a facility certified to be disease-free by Agriculture Canada. The fish were shipped directly to the fisheries laboratory at Lakehead University in Thunder Bay, air express. In turn, they were acclimated to dechlorinated and carbon-filtered city tap water for 3 weeks immediately prior to their use at the study site. Upon arrival at the Lakehead facility, the fish were initially held at the same water temperature as present at the Rainbow Springs hatchery

(10°C). The holding temperature was subsequently raised to 15°C at a rate of 1°C per day, whereupon, the fish were then held for a 2-week period at 15°C. The acclimation water was provided from a once-through delivery system, with a flow rate in excess of 0.7 litres per gram of fish per day. The mean fish weight and length was 5.4 g +/- 0.9 g and 70 mm +/-5 mm, respectively. The university facility was used for the acclimation because it had similar water quality to that of the Manitouwadge area control site.

The fish were fed trout chow daily, ad libitum, up until 24-h prior to their transport to the study site, whereupon feeding was stopped. On the day of transport, batches of 25 fish were placed into individual food-grade resin polyethylene bags containing 10 litres of 15°C water from the acclimation tank. The bags were tied off under pressure with a pure oxygen head space of approximately 10 litres and placed in 90 litre "picnic" coolers, with 3 bags of fish per cooler. One freezer pack was placed in each cooler of fish to ensure that the 15°C water temperatures were maintained.

b) Fish Cages

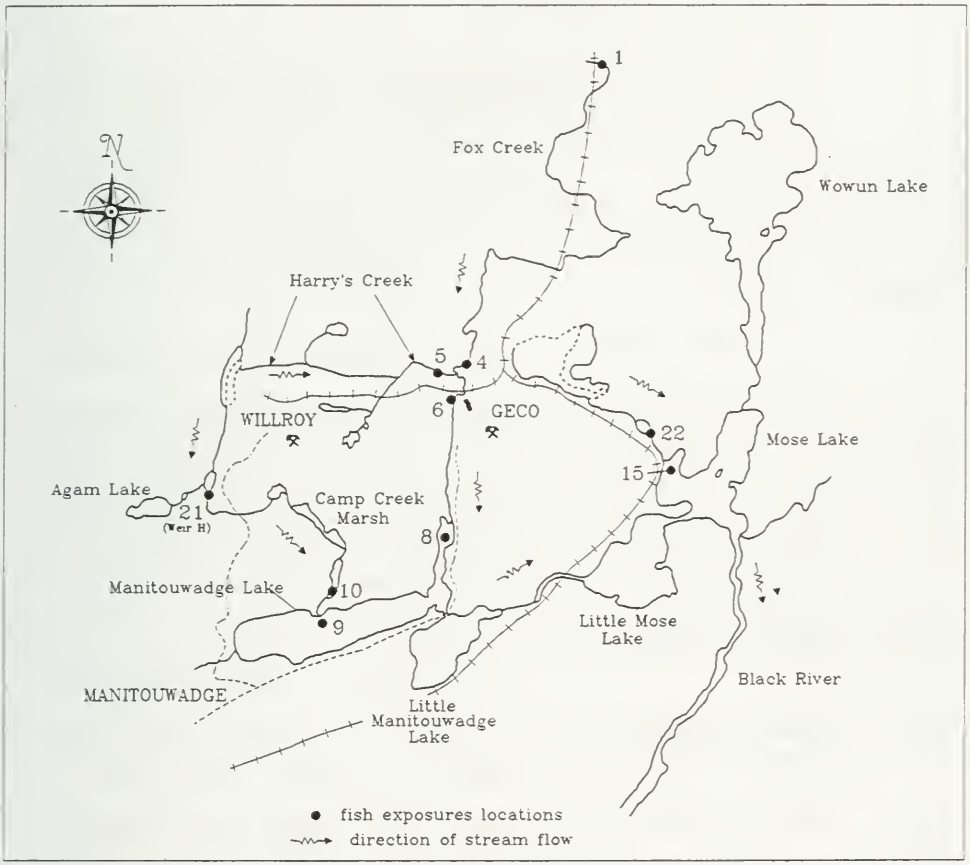
The cages used in the field were constructed with a frame of 1.9 cm. (3/4") polyvinylchloride (PVC) pipe. Their shape was triangular on the top and bottom, and had three sides, each 0.5m in length and depth. The frame supported a 0.6 cm (1/4") mesh seine-net bag, made of nylon, with a "bungee cord" draw-string closure at the top.

A free-floating cage, suspended in the water column with its top at the water surface and held in place by a rope and anchor, was used at each study site. In addition, a submerged cage which was held in place on the bottom by filling the pipe frame with sand, was also used at each of the lake sites. An empty fluorescent-orange plastic container was tied to each cage by rope, in order to serve as a navigational marker, and to assist in retrieving the submerged cages.

3. Cage & Water Sample Locations

Fish were exposed at ten different sites, to monitor mine seepage flows from the Willroy Property through Camp and Harry's Creek, and from the Noranda Geco Mine by way of Fox and Noranda Creeks (Figure 2). The control was located in Fox Creek, upstream of the Harry's Creek inflow. Top- and bottom-exposed cages were located in

FIGURE 2: MANITOUWADGE AREA STUDY SITES.



Lake Manitouwadge at the Camp Creek and Fox Creek inflows, and in Mose Lake at the Noranda Creek inflow, in order to detected possible differences in acute fish lethality at the two depths.

Water samples were collected from each of the fish exposure sites for the first four days of the study, and thereafter, only on days when significant fish mortality (> 20%) was observed at an exposure site, and on the last day of the exposure. Effluent samples were also collected from dam H on the Willroy Property and from the weir at the Geco Mine treatment plant, on study days 1-4 (Sept 16-19th, 1992, inclusive) and on the last day of the exposure (Oct 1st, 1992) for toxicity testing and chemistry analyses.

Specific cage locations were as follows:

Site No.	Name	Description
1	Fox Creek Control:	2nd railroad bridge xing north of Harry's Creek
4	Fox Creek u/s of Harry's Creek:	immediately d/s of small beaver dam - 150m north of Harry's Creek
5	Harry's Creek:	700m u/s of discharge to Fox Cr.
6	Fox Creek d/s of Harry's Creek:	@ abandoned railroad bridge d/s of Harry's Creek
8 *	Manitouwadge Lake @ Fox Cr. discharge:	300m out from Creek mouth, 30m from western shore - depth approx 2m.
9 *	Manitouwadge Lake @ Camp Cr. discharge:	15m out from point of greatest flow from marsh mouth - depth 1.3 m.
10	Camp Creek Marsh:	u/s of dam - 50m from Cr. discharge.
15 *	Mose L. at Noranda Creek:	80m from Cr. discharge, 90m to east-SE shoreline, 175m to nearest part of RR track to the west - depth 2m.
21	Camp Creek d/s of treatment lake weir:	at 1st bend in creek after dam or weir (at seepage H) and before xing

		at Willroy M. road.
22	Noranda Cr. (formerly Muskoka Cr.):	at last dam d/s of effl. treater, on the SW bank prior to marsh & discharge.

* signifies a site at which there was a cage at both the water surface and on the lake bottom.

4. Physical and Chemical Water Sample Analyses

All collections were grab samples, with mercury and other metal samples preserved on site and shipped to the MOEE Thunder Bay lab for analyses. Samples were analysed for a variety of heavy metals (ie. aluminum, cadmium, copper, lead, zinc, chromium, iron, mercury), pH, total ammonium, nitrites, nitrates and total cyanide. Dissolved oxygen, temperature and pH were also measured at each station.

5. Histological Observations

At the end of the 2-week exposure, the live fish at the exposure sites were sacrificed by placing them in a plastic bag and putting it into a cooler of ice until those fish had expired. Within five minutes after having died, those fish to be submitted for histological examination were slit along the belly and placed in a 10% formalin solution for preservation. The samples were shipped air express to Toronto and stored at 4°C. The gill rakers were removed from each fish, fixed in a series of solution dips as described in Appendix A, and stored in wax blocks with three rakers from the left side of each gill arch per block. The blocks were sectioned to 3 um, stained with hematoxylin and eosin and mounted on slides for examination.

Samples were examined with the use of a phase-contrast microscope and they were compared to the Fox Creek control samples.

6. Laboratory Bioassays

Bioassays of grab samples from the Willroy property at the tailings runoff into Camp Creek (dam H) and Noranda Geco Mine effluent were performed at the MOEE Toxicity lab in Rexdale, using rainbow trout (Oncorhynchus mykiss) fry and Daphnia magna neonates.

The rainbow trout for the bioassays at the MOEE laboratory were also obtained from the Rainbow Springs hatchery in Erin, Ontario Their holding temperature was raised from 10°C to 15°C at 1°C

increments per day, three days after arrival at the Rexdale laboratory. After the final holding temperature was attained, the fish were acclimated to the laboratory water for at least two weeks prior to use in the bioassays. The fish were fed Martin's granular trout chow, ad libitum, twice daily during the acclimation period. Feeding was terminated 24 hours prior to use of the fish in the bioassays.

The fish bioassays were aerated 96-h tests. In each bioassay, 6 fish were exposed per test solution and the sample concentrations in each test were 10%, 20%, 30%, 40%, 65%, 100% effluent and a control sample. All dilutions and the control sample were prepared using dechlorinated and carbon-filtered Toronto tap water.

During the initial 24-hour period of the test, fish mortality observations were undertaken at 1, 2, 4, 18 and 24 hours and at least once daily at 48, 72 and 96 hours. All test solutions were kept within a temperature range of 15°C +/- 1°C and dissolved oxygen (DO) levels in those test solutions were maintained above 7.0 mg/L for the duration of the tests. None of the test solutions required pre-aeration prior to the initiation of the test (ie. they had DO > 7.0 mg/L at time of the test setup). Daily measurements of water temperature, dissolved oxygen, pH and conductivity were carried out on subsamples of each test solution.

The neonates (1st instar organisms) used in the Daphnia tests were less than 24-h old and were obtained from a brood stock cultured in the MOEE laboratory. The Daphnia bioassays were 48-h unaerated tests performed at a water temperature of 20°C +/- 1°C. In each bioassay, 12 Daphnia were exposed per test concentration with 3 individuals per test tube, and 4 tubes per test concentration. The test concentrations were 5%, 15%, 30%, 60%, 100% and a control sample. All dilutions and the control samples were prepared using dechlorinated and carbon-filtered Toronto tap water. Measurements of water temperature, dissolved oxygen, pH and conductivity were carried out on subsamples of each test solution at the beginning and end of the test.

Acute lethality results were presented as 96-hour LC50's in the case of the rainbow trout (LC50 being the concentration of a sample which is lethal to 50% of the test organisms within a specified test period) and 48-hour LC50's for the Daphnia bioassays. The LC50's and their confidence levels (fiducial limits) were obtained using the binomial theorem or Spearman-Karber Method of data analysis as outlined in the "LC50 Calculation Program" developed by Charles Stephan of the USEPA (1977). LT50's (time required to produce 50% lethality in the effluent test sample) were determined for the fish bioassay results of the undiluted effluent samples only.

In all of the laboratory bioassays, six (6) fish were used per test solution in order to meet the test loading requirements of at least

0.5 L of effluent/g of fish/day. Those bioassays met the minimum requirement for test fish numbers, as outlined in the former MOEE test protocol (Craig et al, 1983), but not the minimum requirement in the Environment Canada bioassay protocols (Envir. Can. 1990a, 1990b). The reduction in the ideal number of fish from 10 to 6 per test solution allowed for the maintenance of test accuracy at the price of test precision, as outlined on pg. 13, footnote 1, Envir. Canada (1990a). All other procedures met the fish bioassay protocols of the MOEE and Environment Canada (Craig et al, 1983; Envir. Can., 1990a, 1990b).

The Daphnia bioassays met all of the test requirements of the MOEE and Environment Canada bioassay protocols (Poirier et al, 1988; Envir. Can. 1990c, 1990d).

RESULTS & DISCUSSION

1. Surface Water Quality

Heavy rains appear to have increased the leaching of some metals into Fox Creek, immediately upstream of Harry's Creek, before the start of the *in situ* fish bioassay and high metal concentrations showed up most dramatically on pre-exposure day 1 (site 4, Table 1, Figure 2). For example, after the creek levels had receded, iron levels dropped from values of 8500 and 6600 ug/L on the first two days of sampling, to a range of from 1200 - 2800 ug/L, while manganese and zinc concentrations dropped approximately 50 % . The concentrations of copper, lead, cadmium, aluminum, selenium and nickel were not affected by the storm event.

At the same time, all metal concentrations measured in Harry's Creek (site 5, Table 1); including zinc, manganese and iron, were unaffected by the increased rate of leaching from the tailings impoundment on the Willroy property, during the storm event.

The concentrations of copper, zinc, iron, manganese and aluminum at site 6 in Fox Creek between Harry's Creek and the mill complex, were usually greater than levels at the upstream control site (site 4). This increase in metal concentrations was independent of increases in creek flows due to any storm events. Also, regardless of increased flows, a visible plume was always observed in Fox Creek immediately downstream of the Harry's Creek discharge. This plume was likely due to the precipitation of some metals as a result of pH changes at the intersection of the two streams. However, the levels of metals found both up and downstream of Harry's Creek, with the possible exception of the iron concentrations, would not likely cause acute or chronically lethal effects on any life stages of fish.

The main source of metals in Lake Manitouwadge at the mouth of Fox Creek (site 8), appeared to be a number of diffuse sources to the creek, including runoff from the mill complex and from the former "Glory Hole". On study day 1, copper and zinc levels at the mouth of Fox Creek were 14.6 and 4.5 times greater than levels measured upstream of the mill (site 6) during the same sampling run (Table 1). Cadmium, a very toxic heavy metal, also had greater concentrations at the creek mouth relative to levels upstream of the mill, with increases of from 0.7 ug/L to 2.5 ug/L and from 0.5 ug/L to 2.1 ug/L, on study days 1 and 16, respectively, (Table 1). When looking at the day 1 and 16 data, it appears that iron is the only metal that decreased in concentration between Fox Creek upstream of the mill (site 6) and Lake Manitouwadge (site 8), likely by precipitating and settling out as it flowed downstream.

For the two sets of samples involving the Camp Creek (site 9) and Fox Creek discharges to Lake Manitouwadge, metal concentrations

Table 1: Metal levels in water samples collected from fish exposure sites in the Manitouwadge area during the period from Sept. 15, 1991 (PRE-EXP DAY 1) to Oct. 1, 1991 (DAY 16).

DAY No.	METALS (ug/L)	Exposure Sites 1	4	5	6	8	9	10	15	21	22
PRE-EXP DAY 1	Copper	N	0.8*	22.0	4.1	N	N	6.4	N	N	N
	Nickel	O	–	2.1*	1.0*	O	O	–	O	O	O
	Lead	T	3.0*	18.0	5.5*	T	T	–	T	T	T
	Zinc		53	1000	170			34			
	Iron	S	8500	45000	7200	S	S	380	S	S	S
	Manganese	M	120	2100	300	M	M	47	M	M	M
	Aluminum	P	75	3500	400	P	P	20*	P	P	P
	Cadmium	L	–	1.3*	0.4*	L	L	0.2	L	L	L
	Selenium	D	1.0	0.6*	1.0	D	D	0.8*	D	D	D
DAY 1	Copper	1.2*	1.5*	27.0	3.2	47.0	11.0	9.8	6.2	2.4*	50.0
	Nickel	–	–	2.5*	–	2.5*	–	–	1.0*	–	–
	Lead	–	3.0*	19.0	3.5*	5.5*	–	–	2.5*	–	–
	Zinc	2.2*	37	990	140	630	110	38	54	1.3*	130
	Iron	440	6600	49000	6900	2900	370	410	57	270	170
	Manganese	22	93	2200	220	200	36	53	21	4.4*	210
	Aluminum	44	72	3800	290	230	26	25	34	0.7*	200
	Cadmium	–	0.4*	2.5	0.7*	2.5	0.6*	–	–	–	0.3*
	Chromium	–	–	–	–	–	–	–	–	–	–
	Selenium	0.7*	1.0	0.7*	1.0	0.8*	0.7*	0.2*	0.7*	–	0.7*
DAY 2	Copper	0.6*	0.8*	28.0	3.4	N	N	10.0	N	0.9*	52.0
	Nickel	–	–	1.2*	–	O	O	–	O	–	–
	Lead	–	2.5*	18.0	4.0*	T	T	–	T	–	3.0*
	Zinc	1.5*	18	940	140			45		6.7	140
	Iron	480	2000	47000	5200	S	S	570	S	790	180
	Manganese	25	60	2000	210	M	M	56	M	9.9	200
	Aluminum	49	65	3600	320	P	P	28	P	20*	190
	Cadmium	–	0.2*	2.3*	0.5*	L	L	–	L	–	0.5*
	Selenium	0.7*	0.7*	0.7*	0.6*	D	D	0.2*	D	–	0.8*
DAY 3	Copper	N	1.3*	24.0	3.2	N	N	8.4	N	–	50
	Nickel	O	1.0*	1.4*	–	O	O	–	O	–	–
	Lead	T	–	18.0	2.0*	T	T	–	T	–	4.5*
	Zinc		23	950	140			55		6.1	150
	Iron	S	2800	49000	4500	S	S	640	S	610	190
	Manganese	M	63	2000	170	M	M	51	M	9.4	200
	Aluminum	P	65	3700	260	P	P	31	P	150	210
	Cadmium	L	–	1.0*	–	L	L	–	L	–	–
	Selenium	D	0.7*	1.0	0.7*	D	D	0.5*	D	0.7*	0.8*
DAY 4	Copper	3.0	1.6*	27.0	–	N	N	7.6	N	N	N
	Nickel	–	–	1.7*	–	O	O	–	O	O	O
	Lead	3.5*	2.0*	17.0	2.5*	T	T	2.5*	T	T	T
	Zinc	110	24	1000	3.1*			70			
	Iron	5100	2500	53000	460	S	S	970	S	S	S
	Manganese	160	55	2000	21	M	M	59	M	M	M
	Aluminum	270	71	3900	51	P	P	61	P	P	P
	Cadmium	0.6*	0.2*	2.7 U	0.3*	L	L	0.4*	L	L	L
	Selenium	1.0	1.0	0.7*	0.9*	D	D	0.7*	D	D	D
DAY 5	Copper	N	0.7*	N	N	N	N	7.6	N	N	N
	Nickel	O	–	O	O	O	O	–	O	O	O
	Lead	T	–	T	T	T	T	–	T	T	T
	Zinc		23					63			
	Iron	S	2300	S	S	S	S	530	S	S	S
	Manganese	M	52	M	M	M	M	38	M	M	M
	Aluminum	P	70	P	P	P	P	24*	P	P	P
	Cadmium	L	–	L	L	L	L	0.4*	L	L	L
	Selenium	D	0.8*	D	D	D	D	0.5*	D	D	D
DAY 16	Copper	4.5	–	49.0	3.9	45.0	12.0	6.5	5.6	N	N
	Nickel	–	–	2.5*	–	1.5*	–	–	–	O	O
	Lead	4.0*	3.5*	18.0	4.0*	6.0*	3.0*	4.0*	4.0*	T	T
	Zinc	13	14	1100	110	520	130	100	60		
	Iron	260	1200	58000	4200	3300	510	570	80	S	S
	Manganese	14	26	1800	130	97	25	48	43	M	M
	Aluminum	43	51	4400	280	260	30	58	35	P	P
	Cadmium	0.4*	0.3*	2.6**	0.5*	2.1	0.7*	0.3*	0.2	L	L
	Selenium	0.4*	0.8*	0.8*	0.9*	0.9*	1.0	1.0	0.9*	D	D

Note: "–" denotes no measurable response (< detection level).

* denotes a measurable trace amount, interpret with caution.

** denotes an unreliable value,interference during analysis is suspected.

Mercury was not detected at any sites, on the above study days; except at Site 1 on DAY 16 where the level was 0.01 (a trace amount).

Met_Site.wk1

were lower in the lake at the Camp Creek discharge (site 9 vs site 6) by up to 4X for copper, 2X for lead, 5X for zinc, 7X for iron, 5X for manganese, 9X for aluminum and 4X for cadmium (Table 1). In combination with the higher water flow rates from Fox Creek, it is clear that the bulk of the metal loads to the lake are due to the Geco mine/mill complex.

The future MISA regulation for metals in mining effluents state that concentrations for nickel, copper, lead, and zinc should not exceed 0.5, 0.3, 0.2 and 0.5 mg/L, respectively (MOEE, 1994). These limits were not exceeded in any of the samples (N=3) collected from Noranda Creek. In fact, these limits were met at all sites except for zinc in the Harry's Creek effluent (940-1100 ug/L, N=6) and zinc concentrations of 520 and 630 ug/L in two Lake Manitouwadge samples, from the mouth of Fox Creek (Table 1).

Water quality at the various study sites is presented in Appendix B.

2. In-situ Bioassays

a) Acute Fish Lethality.

All of the fish died in Noranda Creek, Harry's Creek and at weir H on the Willroy property, within the initial 24 hours of the exposure (Table 2). Those effects could be directly related to pH with levels of 3.0 and 11.7 measured in Harry's Creek and Camp Creek downstream of the weir, respectively. On the other hand, the lethality in Noranda Creek was likely due to excessively high un-ionized ammonia levels being discharged in the effluent from the Geco mine (Appendix B).

Fish mortality at the other exposure sites appeared to be sporadic throughout the 16-day study (Table 2). The receiving water site with the most mortality during the study was the bottom cage located at a depth of 2.5m in Mose Lake, and approximately 80m out from the Noranda Creek discharge. There was 32% mortality in the bottom cage at that site, while there was only 4% mortality for the surface-water cage at the same location. The mortality occurred over an extended period from study day 4 through to day 16. Oxygen levels at that site appeared to be more than adequate to sustain the bioassay fish during a 2-week exposure, with a level of 11.1 mg DO/L measured at the top and bottom cage, on the last day of the study (Appendix C). Previous studies of the lake have found that a chemocline exists in the lake at a depth of approximately 6m, below which anoxic conditions occur.

The lethality results that were observed in Mose Lake at Noranda Creek are most likely due to either a long-term exposure to chronically lethal levels of ammonia from the sediments and/or the Geco effluent, to a direct exposure to potent metal levels in the

Table 2: Fish Mortality at the various Exposure Sites in the Receiving Waters of the Mine Tailings Effluents during the period from September 15th, 1991 (Day 1) and to October 1st, 1991 (DAY 16).

DAY	Fish Mortality observed at each of the Exposure Sites (A).												
No.	1	4	5	6	8	8-B	9	9-B	10	15	15-B	21	22
DAY 1			25									25	25
DAY 2									1				
DAY 3		4											
DAY 4		1									1		
DAY 5													
Day 10						3	2	1			4		
DAY 16				5	1						2		
Total A	0	5	25	5	1	3	2	1	1	0	7	25	25

	Live Fish remaining at each of the Exposure Sites at the end of the Study (B).												
	1	4	5	6	8	8-B	9	9-B	10	15	15-B	21	22
Day 16	25	17	0	12	22	21	7	23	19	24	17	0	0

	Fish unaccounted for at the end of the Study (C = 25 – {A + B}).												
	1	4	5	6	8	8-B	9	9-B	10	15	15-B	21	22
Day 16	0	3	0	8	2	1	16	1	5	1	1	0	0

	Fish unaccounted for at the end of the Study as a % of Fish that died in the 1st 96 hours (D = C/A).												
	1	4	5	6	8	8-B	9	9-B	10	15	15-B	21	22
Day 16	0/0	3/5 (60%)	0/0	8/5 (160%)	2/1 (200%)	1/3 (33%)	16/0	1/1 (100%)	5/1 (500%)	1/0	1/7 (14%)	0/0	0/0

	% Fish Mortality observed at each of the Exposure Sites during the study (E). *												
	1	4	5	6	8	8-B	9	9-B	10	15	15-B	21	22
Day 16	0	32 %	100 %	> 19 % < 53 %	> 3 % < 13 %	16 %	?	8 %	> 3 % < 25 %	4 %	32 %	100 %	100 %

Note: * the fish that were missing at the end of the study (C) were considered to have died and therefore were used in the calculation of the % mortality (E) - if - the number of unaccounted-for-fish at the site in question at the end of the exposure was less than or equal to the number of fish which died during the 1st 96h of the exposure (ie. D = 100% or less).

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sediments and their interstitial waters, or a combination of several sources of lethality. Bedard and Petro (1994) found copper, zinc, iron and aluminum levels in the sediments at that site of 2200 ug Cu/g (ppm), 4900 ug Zn/g, 78,000 ug Fe/g and 19,000 ug Al/g. Sediment bioassays of those samples at the MOEE Rexdale lab produced 100% mortality to the fathead minnow test organisms over a 21-day exposure period, while mayfly and chironomid mortality was 7% and 18%, respectively (Bedard & Petro, 1994). Control bioassays using clean sediments produced 0 and 18% mortality in the mayflies and choronomids, respectively, and no mortality in the fathead minnows. Therefore, when the mayfly and choronomid data for the Mose Lake sample are normalized relative to the control mortality levels, there is considered to be virtually no invertebrate mortality in the Mose Lake sediment sample.

These results suggest that some toxicant(s), other than metals, may have caused the lethality in the Mose Lake sediment bioassays, for the invertebrates are usually more sensitive to metals than are fish, while the inverse is true for ammonia (Petro, pers. comm.) Ankley et al (1990) identified ammonia as an important sediment-associated toxicant in studies of the Fox River in Wisconsin and suggested that those results had implications for sediment toxicity assessment in other freshwater systems. Ammonia was not measured in the pore water of sediment samples collected during the Manitouwadge study, and therefore further bioassays (field and lab) and sampling of sediments for potential toxic components (such as ammonia) and ancillary constituents which influence their effects would be necessary in order to determine the principal toxic component(s) in those samples.

The water quality at the sediment/water interface in Mose Lake at the mouth of Noranda Creek is considered to be marginally lethal to the exposure fish in that less than 50 % of the fish died in the in situ bioassay at that site. Therefore, un-ionized ammonia concentrations which were extremely lethal to fish at the beaver dam in Noranda Creek (100 % mortality within 24 h); likely dissipated, were transformed or diluted significantly between the dam and the Mose Lake exposure site.

In regard to the lethality results for the Mose Lake surface cage, un-ionized ammonia levels measured on study days 1 and 16 were .097 mg/L and .054 mg/L (Appendix B & C), which are lower than levels cited in the literature as being lethal to fish, and as expected, there was only 4% mortality during that fish exposure.

Ammonia levels measured in the surface waters of Mose Lake were not a problem in regard to fish lethality during this study. However, lethality due to ammonia could have occurred in the lake under certain environmental conditions. For example, the un-ionized ammonia concentration of .054 mg/L, measured on day 16 at the Mose Lake site, was present at a water temperature of 6.1°C (Appendices B & C), while the value would have been 0.19 mg/L at a temperature

of 23°C. Such a level exceeds the lowest level cited in the literature as being acutely lethal to fish over either a 24- or 96-hour exposure period (USEPA, 1985). Furthermore, a water sample collected at the pulp and paper road crossing of the Black River, 1.6km below the Mose Lake outlet (on July 6th, 1987), would have had an un-ionized ammonia concentration of 0.24 mg/L. That value is based on a total ammonia level, pH and water temperature of 4.1 mg N/L, 8.1 (in lab) and 23°C, respectively (MOE, 1991). It should also be mentioned that those may be unusual conditions in that it was a dry summer in 1987, and un-ionized ammonia calculated from data collected once monthly from 1988-93 did not reach levels in excess of the lowest level cited in the literature as potentially lethal to fish (MOEE data base). In the limited number of measurements taken from 1988-93, total ammonia levels exceeded the 4.1 mg/L level cited above, on two occasions; however, lower water temperatures limited the amount of un-ionized ammonia present in those samples. Nevertheless, under extreme conditions, ammonia levels throughout much of the surface waters of the lake could have reached levels in the summer which are potentially lethal to fish. An indication of the potential for fish lethality in Mose Lake water was demonstrated in toxicity studies by Beak (1993). They found that 15% of the fathead minnows died in a 7-day growth test of full-strength samples collected from Mose Lake Narrows.

In conclusion, although elevated ammonia levels produced acutely lethal conditions for fish in Noranda Creek and provided an unknown contribution to the acute lethality observed in Mose Lake, the possible influence of ammonia on the biota of the lake will be greatly diminished with the shutdown of the Geco operation. On the other hand, the toxic effects of heavy metal loadings to the lake will likely last for a much longer period of time.

b) Metal Bioconcentration

The levels of copper, manganese, cadmium and zinc in rainbow trout exposed in Lake Manitouwadge at the Fox Creek discharge were significantly greater ($p < 0.05$) than levels found in fish from each of the other test sites, including the Fox Creek control site, after a 15-day exposure (Table 3). In fact, the concentrations of metals found in the fish from Lake Manitouwadge at Fox Creek were significantly greater than levels in fish exposed in Mose Lake at the Noranda Creek discharge even though metal levels in the sediments of both sites were quite similar. The values (in ug/g) of copper, manganese, cadmium, iron, zinc and aluminum found in the Lake Manitouwadge sediments (with Mose Lake samples shown in brackets) are as follows - Cu: 1,700 (2,200); Mn: 980 (320); Cd, 34 (23); Fe: 68,000 (78,000); Zn: 6,600 (4,900) and Al: 13,000 (19,000) - Bedard & Petro (1994).

We can offer no definitive reason for the differences in the bioavailability of metals to the fish at the mouths of Fox and

Table 3: Metals bioconcentrated in rainbow trout exposed in the Manitouwadge area for a 14-day period from September 15th to October 1st, 1991.

Site		Metal	Metal Concentrations (ng/g) in Fish Sample #					Mean [St. Dev.]* or
No.	Description	Species	1	2	3	4	5	Average (Range) **
C-1	Fox Creek control	copper	0.89	2.4	1.7	1.5	0.82	1.46 [0.65]
		nickel	< 0.30	0.4	< 0.4	0.41	0.33	.34 [0.11]
		zinc	25	30	32	42	25	30.8 [6.98]
		manganese	4.9	3.1	3.7	4	3.1	3.76 [0.75]
		cadmium	0.053	0.055	<.04	<.04	<.03	.033 [.02]
		mercury	0.04	0.02	0.02	0.02	0.02	.024 [.009]
2	Fox Creek U/S of Harry's Cr.	copper	1.3	0.8	0.89	2.1	0.99	1.22 [.53]
		nickel	0.54	0.44	0.59	0.53	0.5	.52 [.06]
		zinc	23	35	21	30	27	27 [5.6]
		manganese	1.7	2.3	1.8	2.1	4	2.4 [.94]
		cadmium	<.04	<.04	0.044	0.058	0.04	.036 [.016]
		mercury	0.02	0.02	0.02	<.01	0.05	.023 [.016]
4	Fox Creek D/S of Harry's Cr.	copper	1	1.8	1.3	1.4	-	1.4 [.33]
		nickel	0.94	0.73	0.66	0.8	-	.78 [.12]
		zinc	28	46	29	29	-	33 [8.7]
		manganese	1.2	2.9	2.4	2.2	-	2.2 [.71]
		cadmium	0.04	0.04	0.04	0.055	-	.044 [.008]
		mercury	0.02	<.01	<.01	<.01	-	<.01
10	Camp Creek Marsh Dam	copper	1.4	1.6	1.1	1.7	1.2	1.4 [.26]
		nickel	0.67	0.78	<.4	<.4	<.4	.41 [.29]
		zinc	33	23	24	38	22	28 [7.1]
		manganese	1.8	1.9	1.2	2	1.1	1.6 [.42]
		cadmium	<.04	<.04	<.04	<.04	<.04	<.04
		mercury	<.01	<.01	<.01	<.01	<.01	<.01

Table continued.....

Table 3: continued.....

Site No.	Site Description	Metal Species	Metal Concentrations (ng/g) in Fish Sample # 1	2	3	4	5	Mean [St. Dev.]* or Average (Range) **
8	Manitouwadge L. at Fox Creek outlet (top cage)	copper	2.5	2.2	1.5	1	1.2	1.7 [.65]
		nickel	<.4	<.4	0.47	0.53	0.45	.37 [.16]
		zinc	37	36	32	27	27	32 [4.7]
		manganese	4.8	6	2.1	1.9	1.9	3.3 [1.9]
		cadmium	0.081	0.1	0.053	0.048	0.061	.069 [.022]
		mercury	<.01	<.01	<.01	<.01	<.01	<.01
8B	Manitouwadge L. at Fox Creek outlet (bottom cage)	copper	11	6.3	10	7.4	3.3	7.6 [3.1]
		nickel	0.45	1.1	0.45	<.4	0.45	.53 [.34]
		zinc	65	48	63	53	41	54 [10]
		manganese	23	8.8	23	13	5.5	14.7 [8.1]
		cadmium	0.33	0.17	0.29	0.16	0.069	.204 [.106]
		mercury	0.02	0.02	<.01	<.01	<.01	<.01
9	Manitouwadge L. at Camp Creek outlet (top cage)	copper	2.1	1.7	1.7	-	-	1.8 [.23]
		nickel	<.4	<.4	<.4	-	-	<.4
		zinc	34	29	33	-	-	32 [2.7]
		manganese	5.2	6.3	3.6	-	-	5.0 [1.4]
		cadmium	0.11	0.14	0.08	-	-	.11 [.03]
		mercury	<.01	<.01	<.01	-	-	<.01
9B	Manitouwadge L. at Camp Creek outlet (bottom cage)	copper	1.4	1.7	1.3	1.7	2.1	1.6 [.31]
		nickel	<.4	<.4	<.4	<.4	<.4	<.4
		zinc	30	34	39	35	25	33 [5.3]
		manganese	2.3	3	3.1	3.1	2.7	2.8 [.34]
		cadmium	0.062	0.059	0.059	0.049	0.11	.068 [.024]
		mercury	<.01	<.01	<.01	<.01	0.02	<.01

Table continued.....

Table 3: continued.....

Site		Metal	Metal Concentrations (ng/g) in Fish Sample #					Mean [St. Dev.]* or
No.	Description	Species	1	2	3	4	5	Average (Range) **
15	Mose Lake at Noranda Creek outlet	copper	1.9	1.3	1.4	1.4	1.1	1.4 [.3]
	(top cage)	nickel	<.4	0.51	0.64	0.78	0.66	.56 [.22]
		zinc	29	33	31	26	22	28 [4.3]
		manganese	1.9	1.8	2.3	3	1.4	2.1 [.61]
		cadmium	0.076	0.056	0.049	0.052	<.04	.051 [.02]
		mercury	<.01	<.01	<.01	<.01	<.01	<.01
15B	Mose Lake at Noranda Creek outlet	copper	1.7	1.6	3.8	1.1	3	2.2 [1.1]
	(bottom cage)	nickel	<.4	0.46	0.69	0.76	<.4	.46 [.26]
		zinc	34	26	40	27	36	33 [6]
		manganese	2.3	1.2	2.9	2.4	2.2	2.2 [.62]
		cadmium	<.04	<.03	0.085	<.04	<.04	<.04
		mercury	<.01	<.01	<.01	<.01	<.01	<.01

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Noranda Creeks. However, a possiblity for the differences may be due to the fact that metal concentrations in sediments over a small area at a specific site may be quite variable. That is, the sediment samples for the metals analyses were collected in the vicinity of the fish exposure cages rather from right underneath them (the sediment sample from a site consisted of a composite sample made up of equal volumes taken from the top 3-5 cm of 3 separate Eckman samples collected from that site) - (Hitchin, pers. comm.).

Fish exposed at most of the sites did not bioconcentrate any appreciable concentrations of mercury during the study, and the analytical results were provided with the proviso that the concentrations that were found are trace amounts and should be interpreted with caution.

c) Histological Fish Examinations

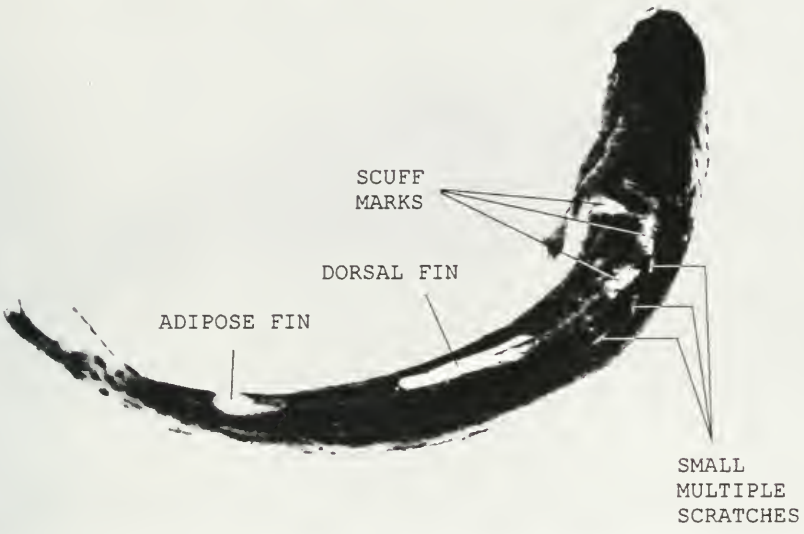
i) Skin

Lesions were observed on the skin of many of our exposure fish in the Manitouwadge study. They appeared, for the most part, as scratches (eg. Camp Creek Marsh - fish #3: top, between dorsal fin and head) or flesh-coloured scuff marks on the skin (eg. Camp Creek - fish #4: abdominal region), (Figure 3). In a few instances, deep, open or ulcerated wounds were observed with a film of blood covering much of the flesh, (eg. Camp Creek Marsh fish #2, Fox Creek, downstream of Harry's Creek, fish #1), (Figure 4). Upon preserving the specimens in a 10% formalin solution, the wounds lost their colour and appeared as bleached out white areas when photographed later. The skin colour of the fish also changed, but instead to a much darker, nearly black shade. An exception in this regard were some samples from the Fox Creek site, downstream of Harry's Creek, which were found dead, and had a more natural colour when preserved. Those fish were probably dead for less than 24 hours, and that concluson is based on the fact that the fish were in good condition and still had significant eye colour when found, rather than being characteristicly opaque a day or two after death.

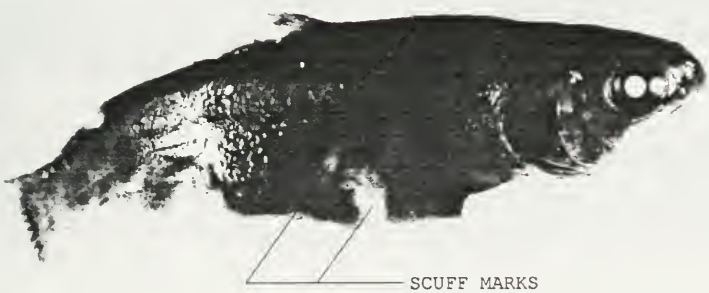
There were only three exposure sites at which skin lesions were not observed on the test fish (Appendix D). The unaffected fish were located at the control site, the Lake Manitouwadge surface cage at Fox Creek and the Mose Lake bottom cage. Fungal infections ("a white fungus growth") and 15-30% mortalities have also been observed in fathead minnows, which were exposed to the Fox Creek control water during a 7-day exposure in the lab (Beak, 1993).

The lesions were found on both bottom-exposed and water column-exposed fish at one of our lake sites (L. Manitouwadge at Camp Creek). All creek exposures except the Camp Creek (marsh site) had the cages resting on the bottom and the fish in 3 of 4 surface

Figure 3 : Skin lesions on exposure fish.

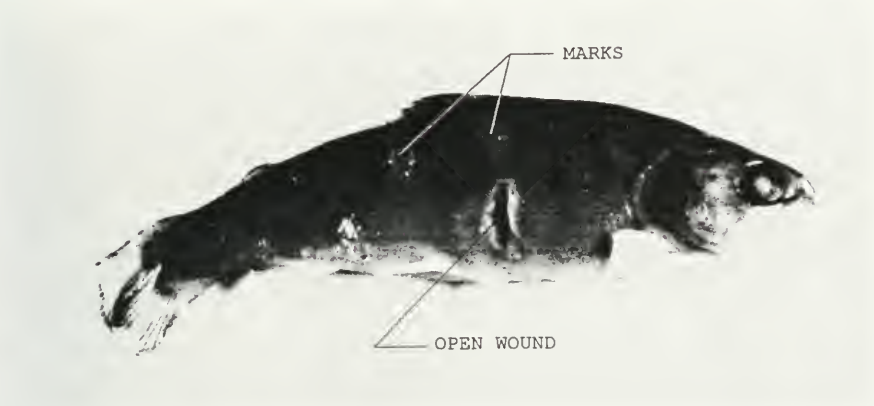


CAMP CREEK MARSH
Fish # 3

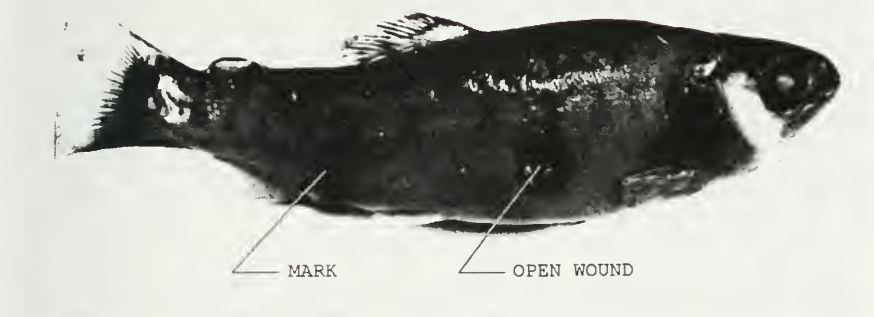


CAMP CREEK MARSH
Fish # 4

Figure 4 : Skin lesions on exposure fish.



CAMP CREEK MARSH
Fish # 2



FOX CREEK - D/S of Harry's Cr.
Fish #1

cages and 5 of 7 sediment cages had lesions by the end of the study. Therefore, lesions were observed on fish which were exposed in at least one of the surface water, stream- or lake-bottom cage locations. The test fish were immature and therefore no differentiation could be made in regard to the prevalence of lesions between sexes. The majority of lesions were straight-line, vertical or diagonal scratches, or openings which occurred through or below the lateral line and posterior to the gillcover and anterior to the anus rather than abrasions (ie. scuff marks). There was one example of "scratches" on the top of a fish, between the head and the dorsal fin. These scratches were not straight lines and had no obvious pattern.

Skin lesions in fish, other than lamprey scarring, have been reported in only a few published field studies. Munkittrick et al (1992), observed lesions in lake whitefish (Coregonus clupeaformis) captured near a bleached, kraft-mill effluent discharge, Reist et al (1987), found that 40 % of the whitefish (Coregonus clupeaformis & C. nasus) sampled during a spawning run in the MacKenzie Delta, N.W.T. had external scars, possibly due to gill nets, or predatory attacks by bears or birds; and Rosen & Hales (1980) concluded that the scarring of many of the paddlefish (Polyodon spathula) caught in the Missouri River was from collisions with powerboat props or attempts of fishermen to snag them.

Munkittrick et al (1992) described the skin lesions, which they found on whitefish, as lateral, slash-like cuts which penetrated to the body cavity of the fish. In the same gill nets, they did not find evidence of lesions, similar or otherwise, on lake trout, lake herring, white suckers, longnose suckers, burbot, northern pike, chinook salmon or walleye. The whitefish lesions may have been due to predatory attack from cormorants, loons or some other fish-eating birds. However, Munkittrick et al did not believe these wounds were consistent with an escape from such prey, in that such wounds would most likely have been more horizontally oriented (Reist et al, 1987). Furthermore, they conclude that the scars were too far posterior to be associated with gillnets and some of the wounds were healed scars suggesting previous injury, while there has been no commercial gillnetting undertaken in the Jackfish Bay area, for many years. In previous work, we have had fish (yearling rainbow trout) which have been attacked by blue herons, and in those cases, all wounds were vertical abrasions which were all above the lateral line and extended up to the upper (cross sectional) arch of the back and were symmetrical on both sides. In this study, lesions were present on both sides of some fish, but the pattern was definitely not symmetrical.

Munkittrick et al (1992) did not find bacterial or fungal growth associated with the lesions, while we did not undertake any such determinations. Pippy and Hare (1969) reported the outbreak of ulcers and open wounds on salmon and suckers after a significant increase of copper and zinc concentrations in the Miramichi River.

They concluded that the stressed fish had died of infection by the pseudomonad bacterium (Aeromonas liquefaciens), a species not previously associated with salmon or sucker mortalities in the wild in North America. It is not known whether Munkittrick et al (1992) checked for Aeromonas. Also, along with the possibility of bacterial infections of fish in our study, there is a chance that the lesions were due to viral attacks on fish which were made susceptible due to the heavy metal exposure. For instance, cadmium has been found to affect the immune system of teleosts (Viale and Calamari, 1984). However, those results were found at exposure levels 100-fold greater than those present at Manitouwadge.

Of the 15 in situ fish exposures that the Standards Development Branch has undertaken throughout Ontario over the last 10 years, this is the first study in which unexplainable skin lesions have been observed on fish held in cages identical to those used in this study. Three of the previous studies involved exposure periods of greater than 96 hours (one for 7 days & the other two for 21 days). The 7-day exposure involved a location downstream of a zinc concentrate spill from a train derailment. Two other studies also had elevated metal levels, one involved an integrated steel mill (96-h exposure), while the other examined organic and inorganic lead from a chemical plant (21-day exposure). Test fish for 6 of the 15 studies were obtained from the same hatchery as those used in this study and the fish for 4 of the studies were acclimated at Lakehead University and transported to the test site in a manner identical to that used in this study.

There were missing fish and/or fish mortalities at all of the sites at which skin lesions were observed and therefore an accurate assessment of the relative occurrence of lesions between sites was not possible.

ii) Gills

It has been demonstrated that heavy metal exposure of fish can cause various degrees of gill damage (Skidmore and Tovell, 1972; Ashley, 1970; Baker, 1969; etc). However, after an extensive statistical review, Mallatt (1985) found that most kinds of gill lesions were often reported in studies with acutely or chronically lethal concentrations, rather than at exposure levels which were chronically sublethal. Studies of heavy metals reported necrosis of the branchial epithelium and hypersecretion of branchial mucous cells, as the most frequent problems (Mallatt, 1985). Other gill damage which was found due to heavy metal exposure are epithelial lifting, lamellar fusion, hypertrophy, and hyperplasia.

Fish can increase their tolerance to certain metals if previously exposed to a sublethal level of that metal over a period of time (copper, Dixon and Sprague, 1981; aluminum, Walker et al, 1991; zinc, Bradley and Sprague, 1991; and metal mixtures, Roch and

McCarter, 1984). In fact, McDonald et al (1990) observed a damage/repair acclimation phenomena during an exposure of fish to aluminum at a pH of 5.2. They observed damage within 24 hours after initiation of the exposure, with a substantial accumulation of aluminum by the gills; and eventual repair was characterized by the elimination of the aluminum. Meuller et al (1990) also demonstrated that gills, which were severely damaged by aluminum, showed a marked improvement in damage by day 14 and were almost totally repaired within 24 days.

Gill samples from fish exposed at all sites except Noranda Creek and Mose Lake were examined for damage that might have been attributed to an exposure to excessive metal concentrations. Although many of the sections contained mild to moderate edema, these results are most likely a fixation problem (ie. mild rotting), as gills are extremely sensitive tissues which need prompt fixation (Smith, memo). Several sections featured hyperplasia from the basal area, a common finding in parasitic infestations, and a parasitic infestation was noted in at least one fish (Fox Creek d/s of Harry's Creek). However, hyperplasia can also occur in areas where there is the deposition of solids (Smith, memo). The Fox Creek fish sample referred to in the last sentence had been dead for approximately 24 hours and had large numbers of parasites attached to the gill lamella. In conclusion, it appears that no significant histopathological changes were observed in the gills of these fish, except for the mild to moderate hyperplasia found in a few scattered samples of fish (Smith, memo).

3. Laboratory Bioassays

a) Acute Fish Lethality.

The Effluent Monitoring Regulations for the Metal Mining Sector require that the Rainbow Trout Acute Lethality Test be undertaken on the effluents of base-metal mines, as of Nov. 24th, 1994. In the Effluent Limits Regulations, an effluent is considered to have failed to comply with the regulation if more than 50% of the rainbow trout die when exposed to undiluted effluent for a 96-hour period (ie. 96-h LC50 < 100%). All of the effluent samples from the Geco Mine and Willroy property at dam H (N=4 each) were lethal to all of the fish exposed in their 100% effluent concentrations (Tables 4 & 5), and as such, those results demonstrate that the effluents would not have met the limits regulation, which is slated to go into effect on August 26th, 1997 (MOEE, 1994).

The LC50 levels for the treated effluent tailings from the Geco Mine ranged from 15.5% to 51% concentrations of effluent: dilution water (v/v) during this study (Table 4). The LC50 results for 5 bioassays of the Geco Mine effluent in 1990 ranged from 14.1% to 27.2%, with the highest 95% confidence level calculated to be 30.1% (Westlake et al, 1992). Three of four Geco Mine effluent samples

Table 4: Laboratory results for rainbow trout bioassays of the Geco Mine final effluent, Manitouwadge, September 16–19, 1991,

Sample Type	Sample Day	Bioassay* LC50 (%v/v)	Effluent Concentration (%v/v)***	% Fish Mortality & (Observation time in h)	Un-ionized Ammonia & (pH) test time = 0–1h	18–24h
Grab	Field Exp. Day 1 (Sept.16)	34.6 (30.0,40.0)	100	100 (1)	10.1(9.1)	
			65	100 (1)	4.6(8.9)	
			40	100 (1)	2.3(8.8)	
			30	0	1.2(8.6)	0.3(8.0)
			20	0	0.5(8.4)	0.2(8.0)
			10	0		
			0	0		
Grab	Field Exp. Day 2 (Sept.17)	51.0 (40.0,65.0)	100	100	12.6(9.4)	9.4(9.2)
			65	100	5.2(9.1)	2.4(8.7)
			40	0	2.2(8.9)	0.3(7.9)
			30	0	1.1(8.7)	0.3(8.0)
			20	0	0.6(8.6)	0.2(8.0)
			10	0		
			0	0		
Grab	Field Exp. Day 3 (Sept.18)	15.5 (13.1,18.3)	100	100	15.7(9.2)	
			65	100	4.1(8.7)	
			40	100	3.1(8.8)	–
			30	100	1.5(8.6)	
			20	83	0.8(8.5)	0.3(8.0)
			10	0	0.4(8.5)	0.1(8.0)
			0	0		
Grab	Field Exp. Day 4 (Sept.19)	42.0 (35.9–49.2)	100	100	8.6(9.1)	
			65	100	4.7(9.0)	
			40	50	1.6(8.7)	
			30	0	1.0(8.6)	0.3(8.0)
			20	0	0.5(8.4)	0.1(7.9)
			10	0		
			0	0		

* Results calculated using the Spearman–Karber Method or Binomial Test Method.

** LT50 – time required to produce 50% fish lethality in the 100% effluent.

*** %v/v – volume of effluent / total test volume, expressed as a percentage.

RBT_EFF.wk1

Table 5: Laboratory results for rainbow trout bioassays of the Dam H runoff at the former Willroy Mine from September 16–19, 1991.

Sample Type	Sample Day	Bioassay* LC50 (%v/v)	Effluent Concentration (%v/v)***	Fish Mortality (%)	LT50 (h)**	pH & (time in h)
Grab	Field Exp. Day 1 (Sept.16)	80.6 (65.0–100.0)	100	100	0.7	11.2 (0); 11.4 (1)
			65	0	–	9.8 (1); 7.5 (21)
			40	0	–	9.4 (1); 7.9 (21)
			30	0	–	9.0 (1); 7.9 (21)
			20	0	–	8.8 (1); 8.0 (21)
			10	0	–	8.4 (1); 8.0 (21)
			0	0	–	8.0 (1); 8.1 (21)
Grab	Field Exp. Day 2 (Sept.17)	51.0 (40.0,65.0)	100	100	9.5	11.8 (0); 11.6 (22)
			65	100	9.5	11.4 (1); 10.1 (22)
			40	0	–	9.8 (1); 7.6 (22)
			30	0	–	9.3 (1); 7.7 (22)
			20	16	–	9.3 (1); 8.0 (22)
			10	0	–	9.0 (1); 8.1 (22)
			0	0	–	8.1 (1); 7.8 (22)
Grab	Field Exp. Day 3 (Sept.18)	51.0 (40.0,65.0)	100	100	0.7	12.0 (0); 11.8 (1)
			65	100	0.7	11.3 (1); 10.7 (18)
			40	0	–	8.8 (1); 7.3 (18)
			30	0	–	9.0 (1); 7.4 (18)
			20	0	–	8.5 (1); 7.6 (18)
			10	0	–	8.6 (1); 7.8 (18)
			0	0	–	8.2 (1); 7.8 (18)
Grab	Field Exp. Day 4 (Sept.19)	51.0 (40.0,65.0)	100	100	0.4	11.9 (0); 11.9 (0.5)
			65	100	0.4	11.3 (0.5); 10.3 (18
			40	0	–	10.0 (0.5); 7.7 (18)
			30	0	–	9.7 (0.5); 7.9 (18)
			20	0	–	9.4 (0.5); 8.0 (18)
			10	0	–	8.7 (0.5); 8.0 (18)
			0	0	–	8.3 (0.5); 8.1 (18)

* Results calculated using the Spearman–Karber Method or Binomial Test Method.

** LT50 – time required to produce 50% fish lethality in a test solution.

*** %v/v – volume of effluent / total test volume, expressed as a percentage.

RBT_DAMH.wk1

in this study were significantly less lethal than the 1990 samples.

The pH levels in the test dilution series of the Geco bioassay samples ranged from 7.8 to 9.4 in this study. The highest pH value at a site for which there was no fish mortality was 8.9, at the start of the (study day-2) bioassay. In turn, that level decreased to 7.9 over the next 24-h period during the test (Table 4).

The Geco effluent appears to have contained lethal levels of un-ionized ammonia. A (study day 1) test solution with an initial un-ionized ammonia concentration of 2.3 mg/L was lethal to fish within the first hour of the exposure (Table 4). However, in contrast, an un-ionized ammonia level of 2.2 mg/L, at the start of the test for the study day 2 sample, produced no fish mortality. Both initial un-ionized ammonia levels (which are calculated values) are well above levels known to be lethal to fish. The difference in lethality of the two samples was likely due to the rate and degree of decrease in the test solution pHs during the first hour or two of the test, and hence the resultant decrease in the exposure levels of un-ionized ammonia. The differences in the rate of the pH decrease may have been due to differences in the alkalinity of each of the samples, but confirmation of such differences was not possible as the lab did not provide water chemistry results for all of the effluent samples.

It was difficult to determine the incipient lethal level (ILL) for un-ionized ammonia in many of the test solutions of the Geco effluent because of the continual decrease in pH and resultant un-ionized ammonia levels during the fish exposure. However, results from the day 3 bioassay did help to better define the ILL. There was an un-ionized ammonia level of 0.8 mg/L in the 20% concentration of the study day 3 bioassay, at the start of the test; and within 30 minutes, 50% of the fish had died in that test solution. After a 24-hour exposure, more of the fish in the 20 % effluent solution had died, to produce a cumulative mortality level of 83 % by that time. The corresponding un-ionized ammonia level had dropped from 0.8 mg/L to 0.3 mg/L during that period. No further drop in pH occurred over the remaining 96 hours and presumably there was no change in the un-ionized ammonia level. Therefore the ILL was likely a value near to, but greater than 0.3 mg/L. Such an un-ionized ammonia level (between 0.3 and 0.8 mg/L) is greater than some concentrations reported in the literature as being lethal to fish.

The un-ionized ammonia level of 0.3 mg/L is also greater than values found in Mose Lake, which had the potential to produce fish mortality. However, a comparison of fish lethality or the potential for fish lethality in the Mose Lake and Geco effluent samples may <u>not</u> be appropriate because of the differences in quality between the dilution waters of the two tests (ie. between the Rexdale lab and the field site dilution waters).

Copper, zinc, iron, manganese, aluminum, cadmium and selenium levels in the Geco Mine effluent were higher than background concentrations at the Fox Creek control (Table 6). Two of the copper levels and the cadmium value on study day 4 had the potential to produce lethal conditions during this study (Table 6). However, elevated hardness values in those samples (Table 7), which ranged from 2030 mg/L to 2780 mg/L (as $CaCO_3$), were well in excess of values required to buffer any potential toxicity from the metal levels found in the effluent.

The most recent bioassays of the Willroy tailings effluent, prior to this study, were performed in 1988 and the LC50's for those two tests were 42% and 97% (Mueller, pers. com.).

In this study, the tailings effluent from the Willroy property had an LC50 of 80.6% for the study day 1 sample and 51% for the day 2 through 4 samples (Table 5). The fish lethality in these samples was due to the high pH values in the effluent, which were a direct result of the effluent treatment process using calcium carbonate ($CaCO_3$). Such an overshot in the pH adjustment is undertaken to ensure that the leaching of metals from the tailings is kept to a minimum. The effects of this alkaline treatment process is buffered approximately 2 km downstream, in the Camp Creek marsh. Therefore, the effect of the tailings discharge on Lake Manitouwadge has been kept to a minimum.

The effluent samples from the Willroy property at dam H had pH values of 11.4, 11.7, 11.6 and 11.5 on study days 1 through 4, respectively (Table 8). The pH of those samples at the start of the bioassays in Toronto was similar to or slightly greater than the values measured in the field (Table 5).

Metal levels in the Willroy samples (Table 9) were much lower than values measured in the Geco Mine samples (Table 6).

b) Acute Daphnia Lethality

The Effluent Monitoring Regulations for the Metal Mining Sector require that the Daphnia magna Acute Lethality Test be undertaken on the effluents of base-metal mines, as of Nov. 24th, 1994. In the Effluent Limits Regulations, an effluent is considered to have failed to comply with the regulation if more than 50% of the Daphnia die when exposed to undiluted effluent for a 48-hour period (ie. 48-h LC50 < 100%). All of the effluent samples from the Geco Mine and Willroy property at dam H (N=4 each) were lethal to all of the Daphnia exposed in their 100% effluent concentrations (Tables 10 & 11), and as such, those results demonstrate that the effluents would not have met the limits regulation, which is slated to go into effect on August 26th, 1997 (MOE, 1994).

In regard to toxic samples from the Metal Mining Sector, approx-

Table 6: Metals in the Noranda Geco Mine effluent, collected at the flume for the period from September 16, 1991 (DAY 1) to October 1, 1991 (DAY 16).

METALS (ug/L)	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 16
Mercury	–	–	–	–	N	–
Copper	130	–	140	35	O	57
Nickel	–	–	–	–	T	–
Lead	–	–	5.5 *	4.0 *		–
Zinc	33 *	1.0 *	30	45	S	49 *
Iron	200 *	180	210	170	A	2300
Manganese	270	2.5 *	230	440	M	210
Aluminum	310	–	110	210	P	290
Cadmium	–	–	–	0.5 *	L	–
Chromium	–	–	–	–	E	–
Selenium	0.9 *	–	–	0.5 *	D	0.9 *

Note: "–" denotes no measurable response (< detection level).

* denotes a measurable trace amount, interpret with caution.

NP indicates the sample was not processed.

These analyses are for subsamples taken from the laboratory bioassay samples.

MET_EFF.wk1

Table 7: Water quality characteristics of the Noranda Geco Mine effluent, collected from the flume during the period from September 16, 1991 (DAY 1) to October 1, 1991 (DAY 16).

Parameter	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 16
Conductivity (μmho/cm at 25°C)	NA	3160	4070	3620		3370
Total Hardness (mg/L as CaCO3)	2370	2030	2780	NP	N	2050
Calcium (mg/L as Ca)	770	660	900	760	O	670
Magnesium (mg/L as Mg)	110	93	130	130	T	91
Sodium (mg/L as Na)	47	64	84	74		49
Potassium (mg/L as K)	54	36	60	48		33
Total Alkalinity (mg/L as CaCO3)	69.5	NP	NP	NP	S	NP
Chloride (mg/L as Cl)	31.0	47	49	48	A	43
Sulphate (mg/L as SO4)	2434	2080	2812	2730	M	2180
Ammonium (mg/L as N)	40.0	31.3	52.5	33.8	P	35.0
Total Nitrates (mg/L as N)	3.18	4.28	3.10	4.95	L	5.00
Nitrite (mg/L as N)	0.45	0.05 *	0.037	0.068	E	0.125
pH	9.5	9.3	9.3	9.0	D	9.2
Total Acidity	NP	NP	NP	NP		NP

Note: NA indicates not analyzed for the parameter.

NP indicates the sample was not processed.

* denotes a measurable trace amount, interpret with caution.

These analyses are for subsamples from the laboratory bioassay samples.

WQ_EFF.wk1

Table 8: Water quality characteristics of the runoff from the Willroy property at Dam H (Site 21) for the period from September 16, 1991 (DAY 1) to October 1, 1991 (DAY 16).

Parameter	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 16
Conductivity (μmho/cm at 25°C)	NA	1470	1510	1400	1420	1030
Total Hardness (mg/L as CaCO3)	530	603	600	ND	580	432
Calcium (mg/L as Ca)	210	240	240	230	230	170
Magnesium (mg/L as Mg)	1.8	1.3	0.5	1.3	0.99	1.8
Sodium (mg/L as Na)	4.6	4.7	4.8	4.9	5.0	3.8
Potassium (mg/L as K)	8.1	8.4	8.4	8.7	9.2	6.4
Total Alkalinity (mg/L as CaCO3)	64.6	152.4	167.1	139.4	130.9	85.7
Chloride (mg/L as Cl)	1.00	1.00	1.00	1.00	1.10	0.84
Sulphate (mg/L as SO4)	467	532	550	577	517	431
Ammonium (mg/L as N)	0.15	0.65	0.12	0.15	0.13	1.03
Total Nitrates (mg/L as N)	0.04 *	0.18 *	0.03 *	0.03 *	0.03 *	0.28 *
Nitrite (mg/L as N)	0.006	–	0.006	0.007	0.008	0.025 *
pH	11.4	11.7	11.6	11.5	11.5	11.4
Total Acidity	NP	NP	NP	NP	NP	NP

Note: NA indicates not analyzed for the parameter.

NP indicates the sample was not processed.

* denotes a measurable trace amount, interpret with caution.

"–" denotes no measurable response (< detection level).

These analyses are for subsamples taken from the laboratory bioassay samples.

WQ_WILR.wk1

Table 9: Metal levels in the runoff from the Willroy property at Dam H (Site 21) for the period from September 16, 1991 (DAY 1) to October 1, 1991 (DAY 16).

METALS (ug/L)	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 16
Mercury	–	–	–	–	NP	–
Copper	–	58	3.1	3.7	0.5 *	–
Nickel	–	–	1.1 *	–	–	–
Lead	–	3.5 *	–	–	–	–
Zinc	–	44	5.1	5.4 *	1.9 *	26 *
Iron	300	160	150	360	90	580
Manganese	–	250	2.8 *	10	1.9 *	17 *
Aluminum	–	280	21 *	47	–	55 *
Cadmium	–	–	–	0.3 *	–	–
Chromium	–	–	–	–	–	–
Selenium	0.3 *	0.3 *	–	0.5 *	0.3 *	0.4 *

Note: "–" denotes no measurable response (< detection level).

* denotes a measurable trace amount, interpret with caution.

NP indicates the sample was not processed.

These analyses are for subsamples taken from the laboratory bioassay samples.

MET_WILR.wk1

Table 10: Laboratory results for Daphnia bioassays of the Dam H runoff at the former Willroy Mine site from September 16–19, 1991.

Sample Type	Sample Day	Bioassay* LC50 (%v/v)	Effluent Concentration (%v/v)***	% Daphnia Mortality & (Observation time in h)	pH at test Start	pH at test End
			100	50 (2)	–	–
Grab	Field Exp.	77.5	100	100 (24)	10.9	9.2
	Day 1	(60.0–100.0)	60	0	9.6	8.0
	(Sept.16)		30	0	9.1	8.2
			15	0	8.5	8.0
			5	8	7.9	7.8
			0	0	7.6	7.6
			100	91 (2)	–	–
Grab	Field Exp.	42.0	100	100 (25)	11.3	9.9
	Day 2	(30.0–60.0)	60	100 (25)	10.8	9.0
	(Sept.17)		30	0	9.3	8.3
			15	0	9.1	8.5
			5	0	8.5	8.2
			0	0	7.7	8.0
Grab	Field Exp.	42.0	100	100 (1)	11.4	10.1
	Day 3	(30.0–60.0)	60	25 (1)	–	–
	(Sept.18)		60	100 (24)	10.9	9.2
			30	0	9.8	8.2
			15	0	9.2	8.4
			5	0	8.7	8.2
			0	0	7.6	7.7
Grab	Field Exp.	42.0	100	100 (1)	11.3	9.0
	Day 4	(30.0–60.0)	60	100 (24)	10.9	8.5
	(Sept.19)		30	0	9.5	8.0
			15	0	9.1	8.1
			5	0	8.5	7.9
			0	0	7.7	7.6

* Results calculated using the Spearman–Karber Method or Binomial Test Method.

** LT50 – time required to produce 50% fish lethality in the 100% effluent.

*** %v/v – volume of effluent / total test volume, expressed as a percentage.

DPH_DAMH.wk1

Table 11: Laboratory results for Daphnia bioassays of the final effluent from the Geco Mine in Manitouwadge, September 16–19, 1991.

Sample Type	Sample Day	Bioassay* LC50 (%v/v)	Effluent Concentration (%v/v)**	% Daphnia Mortality & (Observation time in h)	Calculated Un-ionized Ammonia & (pH) at test Start	End
			100	25 (2)	-	-
			100	100 (24)	11.3 (9.0)	2.9 (8.3)
Grab	Field Exp.	41.0	60	91 (2)	-	-
	Day 1	(30.6,58.1)	60	75 (24)	3.9 (8.7)	1.4 (8.2)
	(Sept.16)		30	0	1.1 (8.4)	0.6 (8.1)
			15	0	0.4 (8.2)	0.2 (8.0)
			5	8	< 0.1 (7.8)	< 0.1 (7.8
			0	0	-	-
Grab	Field Exp.	7.0	100	100	8.8 (9.0)	4.2 (8.6)
	Day 2	(1.5,12.8)	60	91	3.1 (8.7)	1.7 (8.4)
	(Sept.17)		30	75	1.0 (8.5)	0.4 (8.1)
			15	75	0.3 (8.3)	0.2 (8.1)
			5	41	< 0.1 (8.0)	< 0.1 (7.9
			0	0	-	-
Grab	Field Exp.	42.0	100	100	14.8 (9.0)	5.8 (8.5)
	Day 3	(42.0,42.0)	60	100	6.2 (8.8)	2.8 (8.4)
	(Sept.18)		30	0	1.7 (8.5)	1.1 (8.3)
			15	0	0.6 (8.3)	0.3 (8.0)
			5	0	0.1 (8.0)	0.1 (8.0)
			0	0	-	-
Grab	Field Exp.	59.2	100	100	8.0 (8.9)	0.7 (7.7)
	Day 4	(42.3,100.6)	60	0	2.7 (8.6)	0.4 (7.7)
	(Sept.19)		30	41	0.7 (8.3)	0.2 (7.8)
			15	33	0.2 (8.1)	0.1 (7.7)
			5	0	< 0.1 (7.9)	< 0.1 (7.7
			0	0	-	-

* Results calculated using the Spearman-Karber Method or Binomial Test Method.

** %v/v – volume of effluent / total test volume, expressed as a percentage.

DPH_EFF.wk1

imately 50% of the samples were more lethal to trout and 50% are more toxic to Daphnia. However, in some cases, the relative sensitivities of the two test organisms reverses from one sample to the next sample from the same site. Therefore, the MISA Effluent Monitoring document states that, " The toxicity database for the Sector shows that tests of both species are needed to better assess and control the potential impact of these complex and variable effluents on the aquatic environment" (Westlake et al, 1992).

The Daphnia and rainbow trout bioassay results for the treated tailings runoff from the Willroy property were not significantly different. In fact, in each case, when all of the rainbow trout died in a 65 % test solution of a bioassay, there was a equivalent result recorded in the 60 % solution of the same sample in the Daphnia bioassays (Tables 5 & 10). At the same time, there was no test organism mortality in the next lowest test solution in both sets of tests, a 40 % concentration in the case of the rainbow trout test and the 30 % concentration in the case of the Daphnia bioassay. The corresponding pH values measured in the Daphnia bioassays at the start of those tests is provided in Table 10.

In the case of the Geco Mine wastes, results for two of the four Daphnia bioassays (the day 1 & 4 results) were not significantly different from results obtained on those samples when tested using rainbow trout (Tables 4 & 11). However, the day 2 and 3 results for the fish and Daphnia tests were significantly different. We can provide no reason for these differences.

CONCLUSIONS

1. The runoff from the Willroy tailings area to Camp Creek and Harry's Creek was acutely lethal to rainbow trout, most likely due to extremes in pH levels. Harry's Creek had low pH levels, in the range of 3.0-3.5, while high values of from 11.4-11.7 were measured in the Camp Creek samples, collected at dam H. However, the tailings effluent in Camp Creek was adequately buffered by the Camp Creek marsh, so that the final discharge to Lake Manitouwadge was non lethal. Also, the dilution of Harry's Creek at its' confluence with the much greater flows of Fox Creek was sufficient to increase pH and eliminate lethality.

2. There was no significant difference in the uptake of metals by fish exposed in Lake Manitouwadge at the Camp Creek discharge relative to values measured in fish from the Fox Creek control.

3. The Geco mine/mill effluent in Noranda Creek at the last dam prior to discharge into Mose Lake was acutely lethal to rainbow trout. The source of lethality was likely due to excessively high un-ionized ammonia levels.

4. Thirty-two percent of the fish held in direct contact with the sediments of Mose Lake at the Noranda Creek discharge died within 15 days. During the same period, there was only 4 % fish mortality at the water surface of the same site. This fish lethality in the sediment cage was likely due to the deposition of unidentified contaminants from the Geco effluent onto the lake bottom, for the cage position in the water column was not deep enough to have placed the fish in the zone of contaminated water quality under the chemocline of the lake.

5. There was no significant difference in the uptake of metals by fish exposed in Mose Lake at the Noranda Creek discharge relative to values measured in fish held at the Fox Creek control.

6. Ammonia in Mose Lake could have reached levels which were potentially lethal to fish, although other constituents or environmental factors may have buffered those effects.

7. Heavy rains increased the concentrations of some metals (iron, manganese and zinc) leached into Fox Creek upstream of Harry's Creek, while the concentration of other metals was unaffected.

8. There was no evidence of any increase in metal levels or loadings to Harry's Creek as the result of any storm events during the course of the study.

9. Metal concentrations discharged into Fox Creek downstream of Harry's Creek were greater than upstream levels during this study. Furthermore, a distinct plume was observed in Fox Creek immediately downstream of the Harry's Creek discharge, likely due to the precipitation of metals at that point.

10. There were elevated metal levels in Lake Manitouwadge, at the mouth of Fox Creek. The main source appears to be from the mill complex and/or Glory Hole.

11. Fox Creek supplies the bulk of metal loadings to Lake Manitouwadge due to its higher metal concentrations and flow rates relative to other potential sources. However, the Camp Creek discharge continues to make a significant contribution to metal loadings of the lake, even though the Willroy mine has been inactive for many years.

12. The future limits for metals in mining effluents, as outlined in the MISA regulations, were not exceeded in any of the samples from the fully operational Geco mine. In fact, those limits were met at all sites in the study except for zinc in the Harry's Creek effluent (940-1100 ug/L, N=6) and a 520 and a 630 ug Zn/L concentration in two Lake Manitouwadge samples, collected from the mouth of Fox Creek.

13. The bioconcentration of the metals - copper, cadmium, zinc and manganese - by fish exposed on the sediments in Lake Manitouwadge at the mouth of Fox Creek was significantly greater than levels bioconcentrated by fish exposed: i) in the water column (exclusive of the sediments) at the same site, and ii) at the control site.

14. The bioconcentration of the metals - copper, cadmium, zinc and manganese - by sediment-exposed fish in Lake Manitouwadge at the mouth of Fox Creek was also significantly greater than levels bioconcentrated by fish exposed at the water surface or bottom of Mose Lake at the mouth of Noranda Creek, even though the concentration of metals in the sediments at both sites appeared to be similar.

15. Lesions were observed on the skin of fish from a number of exposure sites. No definitive cause could be determined. However, the possibility exists that heavy metal exposure may have affected the immune system of the fish and made them more susceptible to a bacteriological or viral attack.

16. A histological examination did not show any significant pathological changes to the gills of the fish sampled from any of the exposure sites, after a 15-day exposure.

RECOMMENDATIONS

1. The company(ies) which have mined the Willroy and Geco properties in the Manitouwadge area should continue to treat their tailings effluents after the closure of the Geco mine, and until it can be demonstrated to the satisfaction of the MOEE that the effluent from those sources is no longer acutely lethal to rainbow trout, nor would have a detrimental effect on the aquatic biota in the receiving waters of the area.

2. Given the acutely lethal nature of the seepages that originate from the Willroy property, the Company(ies) responsible for environmental compliance should also be required (as part of any final closure plan) to provide the option(s) that they intend to take to address/ameliorate this on-going problem.

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Appendix A: Gill Tissue Preservation Procedure.

Bath Sequence	Solution	Solution Concentration	Bath Time (h)
1	Phosphate-buffered Formalin	10 %	1
2	Ethanol	50 %	1
3	Ethanol	75 %	2
4	Ethanol	75 %	2
5	Phenol in Methanol	8 %	2
6	Phenol in Methanol	8 %	2
7	Ethanol	absolute	2
8	Ethanol	absolute	2
9	Xylene	100 %	2
10	Xylene	100 %	2
11	Wax	100 %	2
12	Wax	100 %	4

Note: Solvent dips undertaken on automatic tissue processor AO T/P 8000.

GIL_PRES.wk1

Appendix B: General Water Chemistry at the fish exposure sites in the Manitouwadge area during the period from September 15th, 1991 (Pre-Exp Day 1) to October 1st, 1991 (Day 16).

DAY No.	Water Quality PARAMETER	Exposure Sites 1	4	5	6	8	9	10	15	21	22
PRE-EXP DAY 1	Conductivity		233	1850	375			552			
	Hardness		126	823	190	N	N	283	N	N	N
	Calcium	N	36	180	48	O	O	100	O	O	O
	Magnesium	O	9	91	17	T	T	8	T	T	T
	Sodium	T	2	8	2			3			
	Potassium		1	9	2	S	S	4	S	S	S
	Acidity	S	21	257	23	A	A	5	A	A	A
	pH	A	7.1	2.9	6.8	M	M	7.8	M	M	M
	Alkalinity	M	82	-	55	P	P	99	P	P	P
	Chloride	P	1	2	1	L	L	1	L	L	L
	Sulfate	L	36	1145	130	E	E	197	E	E	E
	Ammonium	E	0.57	0.91	0.37	D	D	0.02	D	D	D
	Nitrates	D	0.07	0.03	0.06			0.03			
	Nitrites		.018	.118	.018			.003			
DAY 1	Conductivity	185	231	1890	331	327	296	528	846	1680	3500
	Hardness	105	121	832	165	166	137	282	419	627	2270
	Calcium	31	35	180	43	45	41	100	130	250	710
	Magnesium	7	8	93	14	13	9	8	23	1	120
	Sodium	1	1	8	2	3	6	3	14	5	73
	Potassium	.4	1	9	2	2	1	4	9	8	53
	Acidity	5	11	271	24	12	3	6	4	-	8
	pH	7.6	7.2	2.9	7.0	7.4	8.0	7.8	7.9	11.7	8.0
	Alkalinity	97	82	-	62	70	76	91	69	211	44
	Chloride	0.3	0.4	2	1	4	9	1	9	1	35
	Sulfate	1	32	1153	96	87	54	189	379	582	263
	Ammonium	0.03	0.38	0.91	0.37	0.14	0.02	0.02	4.2	0.13	48
	Nitrates	0.04	0.07	0.03	0.07	0.09	0.06	0.02	0.69	0.04	2.9
	Nitrites	.005	.013	.140	.017	.008	.003	.003	.045	.008	.33
DAY 2	Conductivity	185	204	1930	303			534		1330	3400
	Hardness	108	120	874	169	N	N	283	N	604	2180
	Calcium	31	34	200	43	O	O	100	O	240	690
	Magnesium	7	9	91	15	T	T	8	T	1	110
	Sodium	1	1	8	2			3		5	71
	Potassium	1	1	9	1			4		8	52
	Acidity	7	8	265	11	S	S	5	S	-	43
	pH	7.7	7.5	2.9	7.0	A	A	7.8	A	11.5	8
	Alkalinity	97	93	-	68	M	M	90	M	131	8
	Chloride	0.3	0.4	1.9	0.5	P	P	1.2	P	1	33
	Sulfate	1	12	1162	89	L	L	199	L	537	2236
	Ammonium	0.02	0.13	0.89	0.21	E	E	<0.01	E	0.12	34.2
	Nitrates	0.04	0.05	0.03	0.06	D	D	0.02	D	0.04	3.1
	Nitrites	.005	.007	.136	.014			.004		.007	.330
DAY 3	Conductivity		214	1680	290			570		1440	3340
	Hardness	N	Icnc	Icnc	Icnc	N	N	Icnc	N	Icnc	Icnc
	Calcium	O	33	190	40	O	O	100	O	240	680
	Magnesium	T	5	47	15	T	T	8	T	1	110
	Sodium		1	8	2			3		5	70
	Potassium		1	9	1			5		8	51
	Acidity	S	10	242	13	S	S	5	S	-	44
	pH	A	7.5	3.0	7.1	A	A	7.8	A	11.6	7.9
	Alkalinity	M	90	-	72	M	M	87	M	142	8
	Chloride	P	0.4	2	0.5	P	P	1.3	P	1	34
	Sulfate	L	16	1142	72	L	L	211	L	549	2235
	Ammonium	E	0.18	0.84	0.20	E	E	<0.01	E	1	31.8
	Nitrates	D	0.06	0.04	0.06	D	D	0.02	D	0.18	3.28
	Nitrites		.008	.108	.014			.004		.025	.375

WQ_Sites.wk1

continued.....

Appendix B: continued.....

DAY No.	Water Quality PARAMETER	Exposure Sites 1	4	5	6	8	9	10	15	21	22
	Conductivity	296	213	1820	168			586			
	Hardness	154	116	760	98	N	N	304	N	N	N
	Calcium	42	34	180	29	O	O	110	O	O	O
	Magnesium	12	8	76	6	T	T	7	T	T	T
DAY 4	Sodium	2	1	8	1			3			
	Potassium	1	1	9	0.4			5			
	Acidity	9	6	221	5	S	S	2	S	S	S
	pH	7.1	7.4	2.9	7.6	A	A	7.8	A	A	A
	Alkalinity	77	95	–	88	M	M	77	M	M	M
	Chloride	0.5	0.4	1.9	0.4	P	P	1.3	P	P	P
	Sulfate	72	16	1431	2	L	L	232	L	L	L
	Ammonium	0.24	0.19	0.85	0.03	E	E	0.02	E	E	E
	Nitrates	0.06	0.05	0.04	0.04	D	D	0.03	D	D	D
	Nitrites	.011	.008	.126	.007			.003			
	Conductivity		211					617			
	Hardness	N	111	N	N	N	N	306	N	N	N
	Calcium	O	31	O	O	O	O	110	O	O	O
	Magnesium	T	8	T	T	T	T	8	T	T	T
DAY 5	Sodium		1					3			
	Potassium		1					5			
	Acidity	S	6	S	S	S	S	4	S	S	S
	pH	A	7.5	A	A	A	A	7.7	A	A	A
	Alkalinity	M	91	M	M	M	M	72	M	M	M
	Chloride	P	0.5	P	P	P	P	1.2	P	P	P
	Sulfate	L	13	L	L	L	L	232	L	L	L
	Ammonium	E	0.17	E	E	E	E	0.01	E	E	E
	Nitrates	D	0.05	D	D	D	D	<0.01	D	D	D
	Nitrites		.009					.003			
	Conductivity	180	186	1690	263	281	331	634	886		
	Hardness	102	101	666	132	140	150	Icnc	Icnc	N	N
	Calcium	30	29	150	35	38	45	110	130	O	O
	Magnesium	7	7	71	11	11	9	7	23	T	T
DAY 16	Sodium	1	1	7	1	3	6	3	16		
	Potassium	0.4	.5	8	1	1	1.9	5	10		
	Acidity	5	4	231	10	6	3	4	4	S	S
	pH	7.7	7.6	3.1	7.1	7.4	8.0	7.7	7.9	A	A
	Alkalinity	91	86	–	69	74	79	53	71	M	M
	Chloride	0.4	0.4	1.7	0.5	2.6	8.7	1	9	P	P
	Sulfate	2	8	953	56	59	70	258	392	L	L
	Ammonium	0.01	0.08	0.74	0.12	0.11	0.03	0.01	5.04	E	E
	Nitrates	0.06	0.07	0.05	0.08	0.09	0.07	0.03	0.54	D	D
	Nitrites	.004	.005	.040	.013	.009	.003	.003	.036		

Note : * denotes a measurable trace amount, interpret with caution.

** denotes an unreliable value,interference during analysis is suspected.

Icnc denotes "lab could not calculate".

Mercury levels were not detected at any sites, on the above study days; except at Site 1 on DAY 16 where the level was 0.01 (a trace amount).

WQ_Site.wk1

Appendix C: Water Temperature (C), Dissolved Oxygen (mg/L) and pH at the fish exposure sites in the receiving waters of the mine Tailings Effluents during the period from September 16th, 1991 (DAY 1) to October 1st, 1991 (DAY 16).

DAY No.		Water Quality measured at each of the Exposure Sites.														
		1	4	5	6	8	8-B	9	9-B	10	15	15-B	21	22	GM	WP
DAY 1	DO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Temp	15.0	16.0	17.0	16.0	-	-	-	-	-	16.5	-	-	-	-	-
	pH	7.5	7.1	3.1	7.4	-	-	-	-	-	-	-	-	-	-	-
DAY 2	DO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Temp	-	-	14.0	14.0	-	-	-	-	14.0	-	-	16.0	15.0	-	16.5
	pH	-	-	3.0	7.0	-	-	-	-	9.3	-	-	11.6	8.1	9.1	11.7
DAY 3	DO	-	10.2	11.0	13.5	-	-	-	-	11.7	-	-	12.0	11.0	13.0	-
	Temp	-	13.0	11.5	13.0	-	-	-	-	12.0	-	-	15.0	12.5	12.5	-
	pH	-	7.3	3.2	7.2	-	-	-	-	8.0	-	-	11.3	8.1	9.1	-
DAY 4	DO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Temp	-	11.0	10.0	11.0	-	-	-	-	9.0	-	-	-	-	13.5	11.4
	pH	-	7.4	3.4	7.4	-	-	-	-	7.9	-	-	-	-	9.3	11.0
DAY 9	DO	9.2	8.9	-	9.0	9.2	9.2	9.3	-	8.2	-	-	-	-	-	-
	Temp	7.5	7.5	-	7.5	9.0	9.5	13.5	-	7.5	-	-	-	-	-	-
	pH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DAY 16	DO	9.9	10.8	-	10.2	10.8	11.0	10.3	10.3	11.2	11.1	11.1	-	-	10.0	-
	Temp	6.8	5.8	-	6.8	6.0	5.8	9.0	9.0	5.2	6.1	6.1	-	-	10.2	-
	pH	-	-	-	-	-	-	8.4	-	-	-	-	-	-	-	-

NOTE: GM denotes Geco Mine sample collected at the flume.

WP denotes Wilroy Property sample collected at the weir.

Site_DO.wk1

Appendix D: Skin Lesions observed on fish exposed at the various Exposure Sites in the Receiving Waters of the mine Tailings Effluents during the period from September 16th, 1991 (DAY 1)to October 1st, 1991 (DAY 16).

DAY No.	Number of Fish with Skin Lesions* at the end of the Study relative to the number of Remaining Fish.												
	1	4	5	6	8	8-B	9	9-B	10	15	15-B	21	22
Day 16	0/24	3/17	-	4/17	2/22	4/21	0/7	5/23	8/19	7/24	0/19	-	-
	Number of Fish with Skin Lesions* as a % of Fish remaining at the end of the Study.												
	1	4	5	6	8	8-B	9	9-B	10	15	15-B	21	22
Day 16	0	18 %	-	24 %	9 %	19 %	0	22 %	42 %	29 %	0	-	-

Note: * Lesions are considered to be any unusual markings on the fish skin such as scratches on the skin scuff marks or open wounds.

SKIN_LES.wk1